

HIGH PREVALENCE OF AUTOANTIBODIES AGAINST MONOMERIC C REACTIVE PROTEIN (CRP) IN CHILDREN WITH PFAPA SYNDROME

VISOKA PREVALENCA AUTOANTITELA ZA MONOMERNI C REAKTIVNI PROTEIN (CRP) KOD DECE SA PFAPA SINDROMOM

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Summary

PFAPA (periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis) syndrome is an autoinflammatory disorder of unknown etiology. The aim of our study was to evaluate whether the presence of anti-mCRP autoantibodies (anti-mCRP) might possibly contribute to systemic inflammation during PFAPA flares. We carried out anti-mCRP testing (in-house ELISA) in a single-center, prospective cohort of 30 PFAPA patients (12 girls). We found a high prevalence (43.3%) of anti-mCRP antibodies in PFAPA patients during their febrile episodes, which implies the possible involvement of anti-mCRP antibodies in PFAPA pathogenesis.

Keywords: anti-CRP, PFAPA, periodic fevers, autoimmune diseases

Kratik sadržaj

PFAPA (periodične groznice, aftozni stomatitis, faringitis, cervikalni adenitis) sindrom je autoinflamatorno oboljenje sa nepoznatom etiologijom. Cilj ove studije bio je da se utvrdi da li prisustvo anti-mCRP autoantitela (anti-mCRP) možda doprinosi sistemskoj inflamaciji tokom napada PFAPA. Sproveli smo anti-mCRP testiranje (interno sa ELISA) u jedno-centričnoj prospektivnoj kohorti od 30 pacijenata sa PFAPA sindromom (12 devojčica). Otkrili smo visoku prevalencu (43,3%) anti-mCRP antitela kod obolelih od PFAPA tokom febrilnih epizoda, što ukazuje na potencijalno učešće anti-mCRP antitela u patogenezi PFAPA.

Ključne reči: anti-CRP, PFAPA, periodične groznice, autoimune bolesti

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Nonstandard abbreviations: anti-mCRP (autoantibodies against monomeric C reactive protein).

PFAPA (periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis) syndrome is a periodic disease manifesting as recurrent episodes of systemic inflammation, characterized by high fever, cervical adenitis, pharyngitis and aphthous stomatitis (1). PFAPA is generally considered an autoinflammatory disease of compound etiology and heterogenous inheritance, but the exact pathogenesis and the underlying genetic variation remain unclear (2–5).

Monomeric CRP (mCRP) is an isomeric form of CRP with distinct antigenic and physiologic features. It has been suggested that anti-mCRP autoantibodies (anti-mCRP) interfere with mCRP's anti-inflammatory effect activity (clearance of immune complexes and apoptotic debris, complement-modulating effect) leading to an excessive inflammatory response (6–10). The presence of autoantibodies against mCRP has been detected in patients with systemic lupus erythematosus and other autoimmune diseases such as systemic scleroderma, rheumatoid arthritis, Sjögren's syndrome, autoimmune hepatitis, primary biliary cirrhosis, systemic vasculitis and TINU syndrome (8–16). We speculated that anti-mCRP might be present in patients with PFAPA syndrome and might be involved in the pathogenesis of PFAPA febrile flares. The aim of this study was to assess the prevalence of anti-mCRP in PFAPA patients during their febrile episodes.

Thirty children diagnosed with PFAPA syndrome in the Department of Paediatric Infectious Diseases of Wrocław Medical University participated in the study. The diagnostic criteria were detailed in our previous report (17). The presence of anti-mCRP was tested with the use of in-house ELISA as described in the literature (18). Each sample was measured in quadruplicate, the specific absorbency value was normalized with 100% assigned to the reference high anti-mCRP lupus erythematosus serum value and the results were averaged. The cut-off value of the enzyme-linked immunosorbent assay was set as the mean \pm 2 standard deviations of the 3 repeated measures for wells without serum and was 4% of the reference. Additionally, CRP and ESR were determined as part of a routine work-up. CRP levels were measured with immunoturbidimetric assay (Konelab, Thermo Fisher Scientific) and ESR was determined by standard methods.

The cohort consisted of 18 (60%) boys and 12 (40%) girls with a mean age of 4.3 ± 2.1 years. The mean age at disease onset was 2.0 ± 1.2 . Mean duration of a febrile episode was 6 days with a 4.5-week interval between attacks. All patients presented with at least one of the main diagnostic features: pharyn-

gitis (n=29), cervical adenitis (n=29) and oral aphthosis (n=20). All 3 symptoms were present in 19 patients, 10 children had 2 symptoms, and 1 patient presented with only one main symptom. Additional symptoms such as abdominal pain, arthralgias, skin rash, diarrhea, vomiting or headache were present in the majority (90%) of the patients. None of the patients had been receiving corticosteroid treatment before or at the time of blood sampling. The serum mCRP autoantibodies were detected in 13 patients (43.3%) with PFAPA syndrome during their febrile flares. As previously described (11), there was no association between anti-CRP levels and either CRP or ESR. No significant differences were found in age, gender, duration and frequency of the febrile attacks between those who were positive and negative for anti-mCRP.

We identified a high prevalence of anti-CRP antibodies in a single-center, prospective cohort of PFAPA patients. Our study has several limitations, such as the lack of control group and the single-center design. However, this is the first study to investigate the prevalence of anti-CRP in patients with periodic fever syndromes. Autoinflammatory conditions are disorders of innate immunity, characterized by absence of autoreactive antibodies and antigen-specific T-cells – the usual hallmarks of autoimmunity (3). However, PFAPA inflammatory response also involves Th1-type adaptive immunity (3–5), which dominates in several autoimmune diseases (19). Our results suggest that not only a cell-mediated immune response, but also an autoantibody production may play a role in the pathogenesis of PFAPA, linking the disease to autoimmune disorders. Thus, we further speculate that in terms of etiology, the disease should be placed somewhere in the spectrum between autoinflammatory and autoimmune conditions. Anti-CRP could be a target autoantigen in tonsillar and adenoidal tissues, which are inflamed during PFAPA flares. Considering the anti-inflammatory activity of mCRP (8–10), the presence of anti-mCRP might possibly contribute to a systemic inflammation during PFAPA episodes. Anti-mCRP obstructs the mCRP's complement-inhibitory effect, leading to an excessive complement activation. Involvement of the serum complement in PFAPA pathogenesis (mainly upregulated transcription of complement genes) has been implicated before (4).

In summary, we demonstrate that anti-mCRP are prevalent in patients with PFAPA syndrome. Further clinical and mechanistic studies are needed to verify our findings and evaluate the pathogenic role of anti-CRP in periodic fever syndromes.

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*News from the Society of
Medical Biochemists of Serbia*

XXI SERBIAN CONGRESS OF MEDICAL BIOCHEMISTRY AND LABORATORY MEDICINE AND 14th EFLM SYMPOSIUM FOR BALKAN REGION

Dr Snežana Jovičić

Society of Medical Biochemists of Serbia

The biannual Serbian Congress of Medical Biochemistry and Laboratory Medicine with international participation was held in Belgrade 23 – 25th May. It was organized for the 21st time by the Society of Medical Biochemists of Serbia (SMBS) and University of Belgrade Faculty of Pharmacy, under the auspices of the IFCC, EFLM and Balkan Clinical Laboratory Federation (BCLF), as well as the Ministry of Education, Science, and Technological Development and Ministry of Health of Republic of Serbia. During the opening ceremony, participants were welcomed by the president of the congress' Scientific Board, prof. Nataša Bogavac-Stanojević, and also greeted by the Dean of the University of Belgrade Faculty of Pharmacy, prof. Zorica Vujić, as well as the president of the Pharmaceutical Association of Serbia, prof. Vesna Matović.

Traditionally, on this occasion SMBS handed the Award of the Foundation »Magistra Milica Marković«, presented to medical biochemists or laboratories for promoting the technological and organizational work principles of clinical laboratories, improving the quality of laboratory services, and for promoting the profession. This years award went to the Community Health Center Laboratory in Vršac and its Head, dr Jon Čoban.

Also, SMBS acknowledges special contribution to the promotion of medical biochemistry science and profession with Honorary Diploma. This years laureate was Petnica Science Center, situated near the town of Valjevo, in western Serbia, a unique independent institution dedicated to development of scientific culture, scientific literacy, education and culture, primary among high school and university students, as well as training of teachers in novel techniques, methods, and contents in the field of science and technology. The Director of Petnica Science Center, Mr. Vigor Majić, also received an Honorary Diploma for his life long work and endeavours in this

esteemed and unique institution in the world. Also, Mr. Majić opened the working part of the Congress with his plenary lecture entitled »Where are they? – Fermi Paradox and the Art of Losing Gifted Students«.

The working part of the Congress has started with the 14th EFLM Symposium for Balkan Region.

Prof. Ana-Maria Šimundić, President Elect of the EFLM, opened the Symposium with the perspective on the past, present and future of laboratory medicine. This year's Symposium had a running title »Neighboring Countries: the Same Professional Aim«, and it was dedicated in its first part, to the laboratory medicine achievements in neighboring countries of this region – organization of external quality control in laboratory medicine in Bosnia and Herzegovina (Prof. Jozo Čorić), the results of iodine intake survey in Republic of Macedonia in a 10 years gap (dr Sonja Kuzmanovska), experience with interferences in laboratory assays in Montenegro (dr Najdana Gligorović-Barhanović), survey on heavy metals and trace elements in human breast milk in Turkey (dr Ayşegül Çebi), survey on the role of adipose tissue in maintenance of energy homeostasis in Republic of Srpska, Bosnia and Herzegovina (dr Dragana Puhalo Sladoje). Prof. Evgenija Homšak presented the EFLM project »EFLMLABX«, whose aim is exchanging practice in laboratory medicine throughout Europe. Finally, prof. Zorica Šumarac presented the recommendations of the EFLM Working Group on Preanalytical Phase for venous blood sampling.

The first session of the Congress was dedicated to novel trends in laboratory medicine – opportunities, challenges, and perspectives in advanced lipid and oxidative-stress status testing (prof. Jelena Vekić), applications of six sigma and economy methods in clinical laboratory (prof. Nataša Bogavac-Stanojević), the potential of quantitative PCR in laboratory medi-

cine (prof. Miron Sopić), the role of novel biomarkers in inflammatory lung diseases (dr Jasmina Ivanišević), and qualitative characteristics of high density lipoproteins in children with chronic renal disease (dr Danijela Ristovski-Kornic). Patient-oriented approach in the evaluation of analytical quality in medical laboratories was the topic of the second session, where the new concept in quality management in medical laboratories focused on irregular (individual) analytical errors was presented (prof. Svetlana Ignjatović), with the examples of clinical cases in endocrinology related to irregular analytical errors (prof. Miloš arković), and the causes of these errors were presented and elaborated – biotin (dr Vera Lukić) and antibodies (dr Neda Milinković). The third session dealt with the role of laboratory medicine in personalized medicine – the technique of new generation sequencing (prof. Sonja Pavlović), the significance of studying micro RNA (prof. Barbara Ostanek), the role of pharmacogenomics in cancer management (prof. Marija Hiljadnikova Bajro), and the role of gene polymorphisms for adipocytokines in the risk for colorectal cancer development (prof. Ana Ninić). Factors and risk assessment of metabolic and endocrine disorders were the topic of the final session, presenting the integral endocrinological approach in gestational diabetes management (prof. Đuro Macut), risk assessment of exposure to endocrine disrupting chemicals (prof. Biljana Antonijević), European guidelines for risk assessment in food safety (prof. Ivan Stanković), and telomere length as biomarker of aging, environmental and lifestyle influences (prof. Jelena Kotur-Stevuljević).

The second part of the 14th EFLM Symposium for Balkan Region wrapped the whole event, with the

two-part session entitled »Forum of Young Researchers«, in which PhD candidates at the Department of Medical Biochemistry University of Belgrade Faculty of Pharmacy presented their research. The presentations covered topics from the association of polymorphism of matriptase-2 and HFE mutations with hepcidin and iron status in preterminal and terminal renal failure (Miljan Savković), the role of resistin gene expression and its concentration in coronary heart disease (dr Jelena Munjas), the role of genetic factors in resistance to clopidogrel therapy (dr Dragana Bačković), to the relationship between serum biomarkers of vitamin B12 status and morphometric parameters of leukocytes in patients at increased risk of deficiency (Dragana Totošković). In the second part, the connection of cholesterol homeostasis and cardiometabolic risk was presented (Tamara Gojković), followed with the research on PON1 activity distribution among HDL subclasses in renal pathology (Milica Miljković), qualitative and quantitative characteristics of high-density lipoproteins in non-alcoholic fatty liver disease (Jelena Janać), and closing with the qualitative analysis of LDL and HDL cholesterol in colorectal cancer development risk assessment (Milica Stevanović).

This years' Congress and Symposium had over 250 registered participants from Serbia, Bosnia and Herzegovina, Macedonia, Montenegro, and Turkey. The poster session was very vibrant, with the significant participation of students of medical biochemistry of the Faculty of Pharmacy University of Belgrade, with presentation of their scientific projects. With the inspiring discussions that the lectures provoked, and exchange of experience, we may say that expectations were met and even overcome.



Congress Organising team: Danijela Mijailović, Milica Miljković, Tamara Gojković, Bojana Lugić, Iva Perović Blagojević, Snežana Jovičić, Tamara Stamenković



Prof. Nataša Bogavac-Stanojević, president of the Scientific Board, opening the XXI Serbian Congress of Medical Biochemistry and Laboratory Medicine



Prof. Zorica Šumarac, president of SMBS addressed to the Congress participants



Professor Zorica Vujić, Dean of the Pharmaceutical Faculty greeted to the Congress participants



Professor Vesna Matović, President of the Pharmaceutical Association of Serbia greeted to the Congress participants



Laureat of the Award of the Foundation »Magistra Milica Marković«, dr Jon Čoban, with the President of the Foundation dr Milka Golubović



Mr Ljubiomir Štrbac, director of the Makler Company receiving Award Diploma from Milka Golubović, president of Magistra Milica Marković Foundation



Dr Velimir Marković, Milica Marković son greeted participants



Dr Velimir Marković, Prof. Zorica Šumarac, Mr Ljubomir Štrbac, Prof. Nada Majkić-Singh, Dr Jon Čoban, mr Milka Golubović



Vigor Majić, Director of Petnica Science Center, Valjevo with Nada Majkić-Singh



Vigor Majić, Director of Petnica Science Center, Valjevo with Zorica Šumarac



Prof. Zorica Šumarac (president of SMBS), prof. Nataša Bogavac-Stanojević (president of the Scientific Board of the XXI Congress), laureate of the Honorary Diploma Mr. Vigor Majić (director of Petnica Science Center), prof. Nada Majkić-Singh (executive director of SMBS), Mr. Nikola Božić (program director of Petnica Science Center), dr Snežana Jovičić (president of the Organisational Board of the XXI Congress)



Dr Dragana Puhalo-Sladoje, prof. Zorica Šumarac, prof. Jozo Ćorić, prof. Ana-Maria Šimundić, prof. Evgenija Homšak



Prof. Vesna Spasojević-Kalimanovska, dr Sonja Kuzmanovska, dr Zorica Šumarac, dr Ayşegül Çebi, dr Najdana Gligorović-Barhanović



Students of University of Belgrade Faculty of Pharmacy who presented their work during the Poster session